IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Dong, Zheng Xin		
Serial No.:	10/629,261	ART UNIT:	1654
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Entitled:	Analogues of GLP-1		

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Commissioner for Patents
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DECLARATION OF DR. JOHN E. TAYLOR
UNDER 37 C.F.R. §1.132

I, Dr. John E. Taylor, hereby declare and state that:

1. I am familiar with the subject matter claimed in the above-identified patent application, U.S. Serial No. 10/629,261.

2. I am the Associate Director of Receptor & Cell Biology at Biomeasure, Incorporated, of Milford, Massachusetts. I was awarded a Bachelor of Science degree in Zoology from Brigham Young University of Provo, Utah, in 1971, a Master of Science degree in Pharmacology in 1974 and a Doctorate of Philosophy degree in 1977 from the University of the Pacific of Stockton, California. I have been employed by Biomeasure, Incorporated, from 1983 to the present. Part of my responsibilities as Associate Director of Receptor & Cell Biology is to supervise the performance of receptor binding assays of compounds at Biomeasure, Incorporated.

3. I understand that the Examiner of this application is of the view, as stated in the last office action issued in this application dated August 8, 2006, that in the absence of experimental data supporting the asserted activity of the claimed compounds, claims 1, 3, 4, 9-11, 15, 16, and 19 contain subject matter which is not described in the

specification in such a way as to enable one skilled in the art to which it pertains or with which it is most nearly connected, to make and/or use the invention.

4. I make this declaration to show that data generated by following the procedures disclosed in this application (at pages 14-15), which is also described in paragraphs 5 and 6 below, provide sufficient and convincing evidence that the claimed compounds of this application are specific for the GLP-1 receptors and possess the ability to evoke a GLP-1-like response from cells expressing a GLP-1 receptor. One of skill in the art would readily appreciate that the efficacy of any of the compounds of the invention can be determined by using such standard assays. Thus, a person of skill in the art would have been able to determine the suitability of the compounds of claims 1, 3, 4, 9-11, 15, 16, and 19.

5. **Cell Culture:** cDNA clones of the rat and human glucagon-like peptide-1 receptors (2, 3) were obtained from Dr. Andreas Wilmen, University of Marburg, Germany. The coding regions were cloned into the EcoRI site of the mammalian expression plasmid, pTEJ8 (5). DNA transfection of a Chinese hamster ovary cell line, CHO-K1 (American Type Culture Collection, Rockville, MD), was carried out by the calcium phosphate precipitation method as previously described (6). Clonal cell lines stably expressing the rat and the human GLP-1 receptors were obtained by selection of the DNA-transfected cells in RPMI 1640 medium supplemented with 10% fetal bovine serum, 1 mM sodium pyruvate, 1% non-essential amino acids, containing 0.8 mg/ml G418 (Gibco BRL, Grand island, NY), ring cloned, and expanded in culture.

6. **Radioligand Binding:** Cell membranes were prepared for radioligand binding studies by homogenization of the cells in 20 ml of ice-cold 50 mM Tris-HCl with a Brinkman Polytron (Westbury, NY) (setting 6, 15 sec). The homogenates were washed twice by centrifugation (39,000 g / 10 min), and the final pellets were resuspended in 50 mM Tris-HCl, containing 2.5 mM $MgCl_2$, 0.1 mg/ml bacitracin (Sigma Chemical, St. Louis, MO), and 0.1% bovine serum albumin ("BSA"). For assay, aliquots (0.4 ml) were incubated with 0.05 nM (^{125}I)hGLP-1(7-36)NH_2 (~2200 Ci/mmol, New England Nuclear, Boston, MA), with and without 0.05 ml of unlabeled competing Test Peptide

or Reference Peptide. After a 100 min incubation (25 C), the bound (^{125}I)hGLP-1(7-36)NH_2 was separated from the free by rapid filtration through GF/C filters (Brandel, Gaithersburg, MD), which had been previously soaked in 0.5% polyethyleneimine. The filters were then washed three times with 5 ml aliquots of ice-cold 50 mM Tris-HCl, and the bound radioactivity trapped on the filters was counted by gamma spectrometry (Wallac LKB, Gaithersburg, MD). Specific binding was defined as the total (^{125}I)hGLP-1(7-36)NH_2 bound minus that bound in the presence of 1000 nM hGLP-1(7-36)NH_2 (Bachem, Torrance, CA). Binding data were analyzed by computer-assisted nonlinear regression analysis (Data Analysis Toolbox, v.1.0, Molecular Design Limited, San Leandro, CA) and Inhibition Constant (Ki) values were calculated using the equation of Cheng and Prusoff (Cheng Y., Prusoff W. H., Biochem. Pharmacol. 22: 3099-3108, 1973).

7. **Results:** The Binding Constants for native hGLP-1(7-36)NH_2 and representative compounds of the present application, as determined by the foregoing assay, are as follows:

GLP-1 Analogue	**Ki (nM)**
hGLP-1(7-36)NH_2	1.093
(Aib^{8,35})hGLP-1(7-36)NH_2	0.947
(Aib^{8}, β-Ala^{35})hGLP-1(7-36)NH_2	1.257
(Aib^{8,35}, Arg^{26,34}, Lys^{36}(N$^{\varepsilon}$-tetradecanoyl))hGLP-1(7-36)NH_2	43.539
(Aib^{8,35}, Arg^{26}, Lys^{34}(N$^{\varepsilon}$-tetradecanoyl))hGLP-1(7-36)NH_2	34.973
(Aib^{8,35,37}, Arg^{26,34}, Lys^{38}(N$^{\varepsilon}$-tetradecanoyl))hGLP-1(7-38)NH_2	12.113
(Aib^{8,35}, Arg^{26,34}, Lys^{36}(N$^{\varepsilon}$-decanoyl))hGLP-1(7-36)NH_2	25.847
(Aib^{8,35}, Arg^{26,34}, Lys^{36}(N$^{\varepsilon}$-tetradecanoyl), β-Ala^{37})hGLP-1(7-37)OH	18.850
(Aib^{35})hGLP-1(7-36)NH_2	0.831
(Aib^{8,35}, A6c^{32})hGLP-1(7-36)NH_2	2.261
(Aib^{8,35}, Glu^{23})hGLP-1(7-36)NH_2	1.983
(Aib^{8,24,35})hGLP-1(7-36)NH_2	2.971
(Aib^{8,25,35})hGLP-1(7-36)NH_2	5.060
(Aib^{8,35}, Glu^{23}, A6c^{32})hGLP-1(7-36)NH_2	2.555
(Aib^{8}, Glu^{23}, β-Ala^{35})hGLP-1(7-36)NH_2	2.657
(Aib^{8,35}, Arg^{26,34})hGLP-1(7-36)NH_2	4.967

(Aib^{8}, Arg^{26,34}, β-Ala^{35})hGLP-1(7-36)NH_2	2.080
(Aib^{8,35}, Lys^{26}(N^{ε}-decanoyl), Arg^{34},)hGLP-1(7-36)NH_2	35.168
(Aib^{8,35}, Arg^{26,34}, Lys^{36}(N^{ε}-octanoyl))hGLP-1(7-36)NH_2	28.082
(Aib^{8,35}, Arg^{26,34}, Lys^{36}(N^{ε}-decanoyl))hGLP-1(7-36)OH	24.526
(Aib^{8,35}, Lys^{25}, Arg^{26,34}, Lys^{36}(N^{ε}-decanoyl))hGLP-1(7-36)OH	79.775
(Aib^{8}, β-Ala^{35}, Aec^{37})hGLP-1(7-37)NH_2	9.573
(Aib^{8}, β-Ala^{35}, Aec^{38})hGLP-1(7-38)NH_2	1.693
(Aib^{8}, β-Ala^{35}, Aec^{37,38})hGLP-1(7-38)NH_2	2.340
(Aib^{8}, Arg^{26,34}, β-Ala^{35}, Lys^{36}(N^{ε}-Aec-decanoyl))hGLP-1(7-36)NH_2	15.890
(Aib^{8,35}, Arg^{26,34}, Ava^{37}, Ado^{38})hGLP-1(7-38)NH_2	30.907
(Aib^{8,35}, Arg^{26,34}, Asp^{37}, Ava^{38}, Ado^{39})hGLP-1(7-39)NH_2	14.513
(Aib^{8,35}, Arg^{26,34}, Aun^{37})hGLP-1(7-37)NH_2	31.733
(Aib^{8,17,35})hGLP-1(7-36)NH_2	4.940
(Aib^{8}, Arg^{26,34}, β-Ala^{35}, D-Asp^{37}, Ava^{38}, Aun^{39})hGLP-1(7-39)NH_2	6.528
(Gly^{8}, β-Ala^{35})hGLP-1(7-36)NH_2	4.805
(Ser^{8}, β-Ala^{35})hGLP-1(7-36)NH_2	5.220
(Aib^{8}, Glu^{22,23}, β-Ala^{35})hGLP-1(7-36)NH_2	1.190
(Gly^{8}, Aib^{35})hGLP-1(7-36)NH_2	3.610
(Aib^{8}, Lys^{18}, β-Ala^{35})hGLP-1(7-36)NH_2	4.553
(Aib^{8}, Leu^{27}, β-Ala^{35})hGLP-1(7-36)NH_2	6.755
(Aib^{8}, Lys^{33}, β-Ala^{35})hGLP-1(7-36)NH_2	2.840
(Aib^{8}, Lys^{18}, Leu^{27}, β-Ala^{35})hGLP-1(7-36)NH_2	10.490
(Aib^{8}, D-Arg^{36})hGLP-1(7-36)NH_2	2.546
(Aib^{8}, β-Ala^{35}, D-Arg^{37})hGLP-1(7-37)NH_2	8.390
(Aib^{8,27}, β-Ala^{35})hGLP-1(7-36)NH_2	6.223
(Aib^{8,27}, β-Ala^{35,37}, Arg^{38})hGLP-1(7-38)NH_2	14.613
(Aib^{8,27}, β-Ala^{35,37}, Arg^{38,39})hGLP-1(7-39)NH_2	21.790
(Aib^{8}, Lys^{18,27}, β-Ala^{35})hGLP-1(7-36)NH_2	12.198
(Aib^{8}, Lys^{27}, β-Ala^{35})hGLP-1(7-36)NH_2	9.203
(Aib^{8}, β-Ala^{35}, Arg^{38})hGLP-1(7-38)NH_2	2.700
(Aib^{8}, Arg^{26,34}, β-Ala^{35})hGLP-1(7-36)NH_2	2.080
(Aib^{8}, D-Arg^{35})hGLP-1(7-36)NH_2	5.097
(Aib^{8}, β-Ala^{35}, Arg^{37})hGLP-1(7-37)NH_2	3.953
(Aib^{8}, Phe^{31}, β-Ala^{35})hGLP-1(7-36)NH_2	1.767
(Aib^{8,35}, Phe^{31})hGLP-1(7-36)NH_2	1.390

(Aib^{8,35}, Nal^{31})hGLP-1(7-36)NH_2	2.818
(Aib^{8,35}, Nal^{28,31})hGLP-1(7-36)NH_2	5.613
(Aib^{8,35}, Arg^{26,34}, Nal^{31})hGLP-1(7-36)NH_2	6.193
(Aib^{8,35}, Arg^{26,34}, Phe^{31})hGLP-1(7-36)NH_2	2.117
(Aib^{8,35}, Nal^{19,31})hGLP-1(7-36)NH_2	7.623
(Aib^{8,35}, Nal^{12,31})hGLP-1(7-36)NH_2	4.693
(Aib^{8,35}, Lys^{36}(N^{ε}-decanoyl))hGLP-1(7-36)NH_2	32.787
(Aib^{8,35}, Arg^{34}, Lys^{26}(N^{ε}-decanoyl))hGLP-1(7-36)NH_2	35.168
(Aib^{8,35}, Arg^{26,34}, Lys^{36}(N^{ε}-dodecanoyl))hGLP-1(7-36)NH_2	44.167
(Aib^{8}, β-Ala^{35}, Ser^{37}(O-decanoyl))hGLP-1(7-37)NH_2	29.850
(Aib^{8,27}, β-Ala^{35,37}, Arg^{38}, Lys^{39}(N^{ε}-octanoyl))hGLP-1(7-39)NH_2	43.780
(Aib^{8}, Arg^{26,34}, β-Ala^{35}, Lys^{37}(N^{ε}-octanoyl))hGLP-1(7-37)NH_2	19.170
(Aib^{8}, Arg^{26,34}, β-Ala^{35}, Lys^{37}(N^{ε}-decanoyl))hGLP-1(7-37)NH_2	26.505
(Aib^{8}, Arg^{26,34}, β-Ala^{35}, Lys^{37}(N^{ε}-tetradecanoyl))hGLP-1(7-37)NH_2	37.190
(Aib^{8}, Arg^{26,34}, β-Ala^{35}, Lys^{37}(N^{ε}-dodecanoyl))hGLP-1(7-37)NH_2	17.255
(Aib^{8}, Arg^{26,34}, β-Ala^{35}, Lys^{37}(N^{ε}-dodecanoyl))hGLP-1(8-37)NH_2	502.500

8. **Conclusion:** The results of the Radioligand Binding assay described hereinabove demonstrate that the representative compounds of the present invention bind to the GLP-1 receptor with substantially the same affinity as hGLP-1(7-36)NH_2. Thus, the application supplies sufficient data and information to practice the invention of the claims. In view of the data presented above and the Applicant's comments, it is believed that the Examiner's concern has been addressed.

9. I further declare that all statements made herein of my own knowledge are true and that statements made upon information and belief are believed to be true and further that false statements and the like so made are punishable by fine or imprisonment or both under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the above-identified application or any patent issuing thereon.

2/5/07
Date

John E. Taylor